EXHIBIT 30

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The relative merits and drawbacks of new nucleoside analogues with clinical potential

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A large number of nucleoside analogues have been found to have antiviral activity, mainly against herpesviruses. The involvement of cellular as well as viral enzymes in the mode of action of several nucleoside analogues makes a prediction of clinical efficacy difficult. The possibility and consequences of incorporation into cellular DNA are other important aspects of nucleoside analogues as antiviral drugs. It seems likely that in the next few years enough knowledge about mechanism of action, consequences of incorporation into DNA, efficacy in different test systems and in clinical trials will accumulate to allow an understanding of how to design even better antiviral drugs.

Introduction

In recent years a large number of nucleoside analogues have been described as antiviral agents. Most of these compounds have been active against herpesviruses, partly because herpesviruses have been the main targets for efforts to find antiviral drugs.

It has become increasingly clear that the relative cell culture efficacy of nucleoside analogues against herpesviruses does not reflect the efficacy in infected animals. It is not yet possible to predict clinical efficacy from cell culture data nor is the extent and consequences known of incorporation of nucleoside analogues into cellular DNA. Despite this ignorance it might be worthwhile discussing some of the advantages and disadvantages with nucleoside analogues as antiviral drugs.

Viral enzymes involved in nucleic acid synthesis

One reason why nucleoside analogues have been the most common structural type of antiviral compounds is that most viruses use RNA or DNA polymerases coded for by the viral genome and different from cellular polymerases (Helgstrand & Öberg, 1980). Table I shows that the only viruses not having DNA or RNA polymerases are parvo and papova viruses. Several other enzymes involved in the synthesis of viral nucleic acids are also coded for by the viral genome as indicated in Table II for herpes, influenza and rhino viruses. The herpesvirus thymidine kinase (TK) is a key enzyme for many nucleoside analogues. It is induced by herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and varicella-zoster virus (VZV) but not by Epstein-Barr virus (EBV) or cytomegalovirus (CMV).

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Table I. Virus specific enzymes

Virus	RNA or DNA polymerase	Other enzymes
Picorna	+	+
Reo	+	+
Toga	+	+
Orthomyxo	+	+
Paramyxo	+	+
Corona	+	+
Rhabdo	+	+
Retro	+	+
Arena	+	?
Bunya	+	?
Parvo	?	–
Papova	–	?
Adeno	+	+
Herpes	+	+
Irido	+	+
Pox	+	+
Heptitis B	+	+

Table II. Viral enzymes

Virus	Ribonu-cleotide reductase	Protein kinase	TK	DNA pol	RNA pol	DNase	RNase	Neura-mini-dase	Protease
HSV-1	+	+	+	+	–	+	–	–	–
HSV-2	+	?	+	+	–	+	–	–	–
VZV	?	?	+	+	–	+	–	–	–
EBV	+	?	–	+	–	+	–	–	–
CMV	?	?	–	+	–	+	–	–	–
Influenza	–	+	–	–	+	–	+	+	?
Rhino	–	–	–	–	+	–	?	–	+

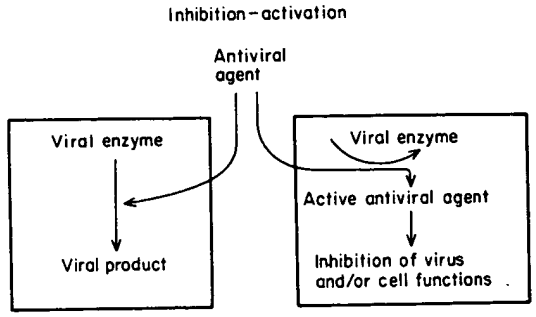


Figure 1. Principal mechanisms of action of antiviral agents, selective inhibition and selective activation.

Activation and inhibition

The viral enzymes can be utilized as targets for antiviral agents in two different ways, sometimes combined consecutively, as shown in Figure 1. A nucleoside analogue, or any other type of inhibitor, could either selectively inhibit a necessary viral function at concentrations not affecting cellular enzymes, or it could be transformed by a viral enzyme into an active inhibitor. If the activation of the compound is a very selective process carried out almost exclusively by a viral enzyme, then the activated compound does not have to be very selective in its further activity. It could block both viral and cellular functions if it is formed only in the infected cell. However, in most cases enzymic reactions are not that selective and it is an advantage if the activated inhibitor also has a selective action against a viral function. The nucleoside analogues we will discuss all have to be activated in several steps by both viral and cellular enzymes and they also interact with viral polymerases.

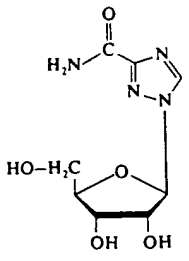
A general property of viral enzymes involved in nucleic acid synthesis is that the structural requirement, as regards substrates, is less strict than the structural requirements of the corresponding cellular enzymes. This is of importance since most nucleoside analogues active as antiviral drugs are substrate analogues.

Selected nucleoside analogues

This is not a complete review of nucleoside analogues with antiviral activity but rather a discussion of the few selected compounds shown in Table III. One of these compounds, ribavirin, has been used against RNA viruses and the other compounds against DNA viruses, mainly herpes viruses.

Table III. Antiviral nucleoside analogues

Ribavirin	Idoxuridine	Bromovinyldeoxyuridine	Acyclovir
Adenine arabinoside	Trifluoro thymidine	Fluro-iodo-arabinofuranosyl-cytosine	Dihydroxybutylguanine Dihydroxypropoxymethyl-guanine



Ribavirin

Figure 2. Ribavirin.

Ribavirin

Figure 2 shows the structure of ribavirin. This ribonucleoside analogue has a modified base which resembles both guanine and adenine as shown for the triphosphates in Figure 3. Phosphorylation to triphosphate is carried out by cellular enzymes and

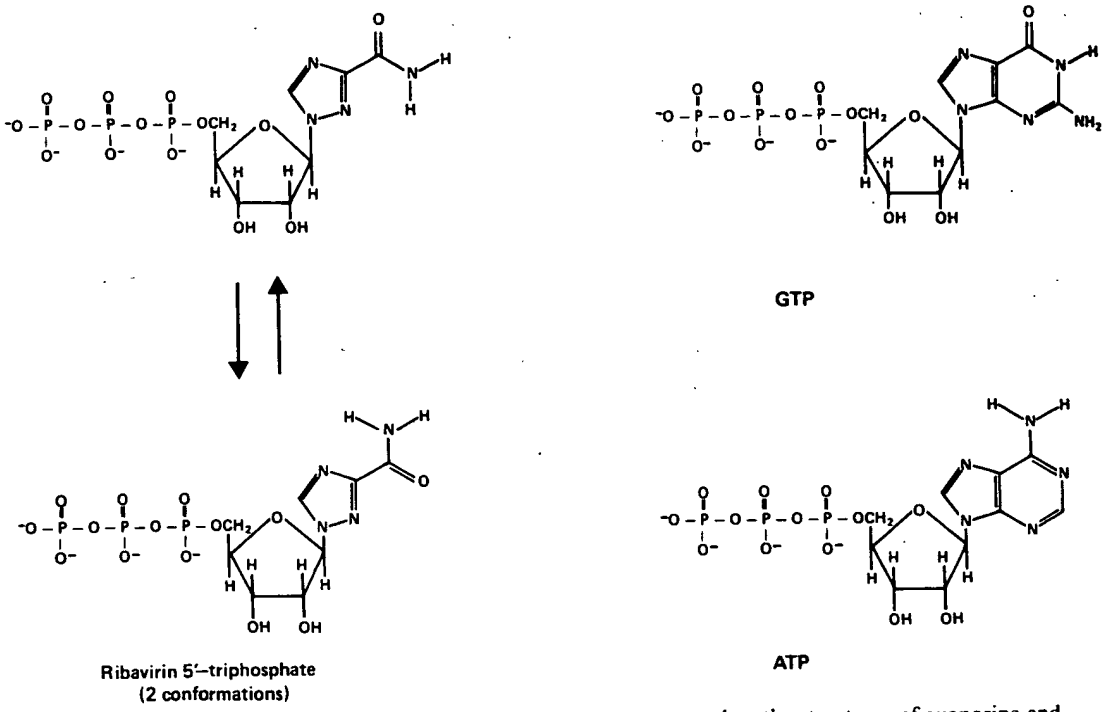


Figure 3. Ribavirin triphosphate, alternative structures compared to the structures of guanosine and adenosine triphosphates.

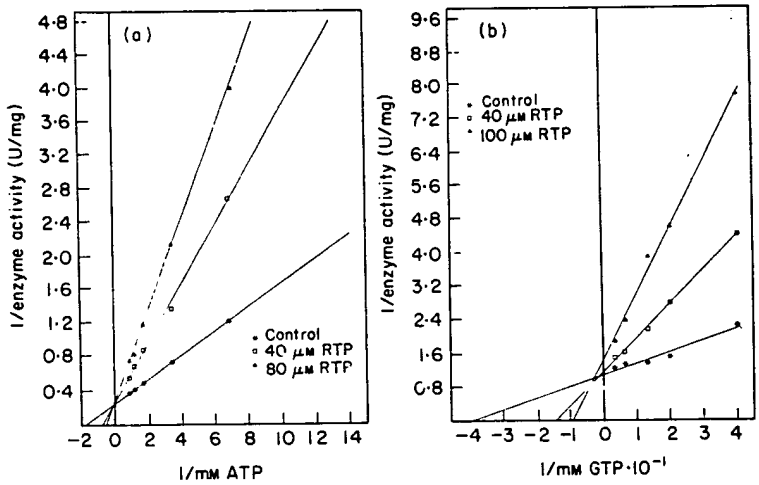


Figure 4. Inhibition of influenza RNA polymerase by ribavirin triphosphate (RTP). From Eriksson *et al.* (1977) with permission.

ribavirin triphosphate inhibits influenza RNA polymerase. In a cell-free assay this inhibition is competitive with respect to GTP and ATP as shown in Figure 4, but not with the other triphosphates, in accordance with its structural features (Eriksson *et al.*, 1977). In cell culture and *in vivo* it is likely that the inhibition of influenza virus replication is mediated also by mechanisms other than by inhibition of the viral

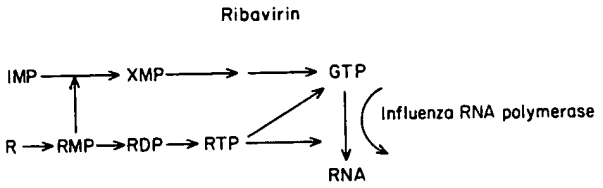


Figure 5. Ribavirin, mechanisms of action. Ribavirin monophosphate (RMP) inhibits IMP dehydrogenase, it also competes with GTP in the polymerase reaction and it affects the formation of cap structures on cellular mRNA required as primers for the influenza polymerase reaction. IMP, Inosine monophosphate; R, ribavirin; XMP, xanthine monophosphate.

Table IV. Clinical effect of ribavirin aerosol against influenza A

	Day 0	Day 1	Day 2
Systemic illness			
Treated	2·64	1·50	1·0
Control	2·56	2·18	1·38
P	NS	0·004	0·07
Rhinitis, pharyngitis, tracheobronchitis			
Treated	1·52	1·31	0·77
Control	1·49	1·31	1·14
P	NS	NS	0·07
Temperature			
Treated	39·4	38·6	36·9
Control	39·2	38·6	37·4
P	NS	NS	0·003

Patients were treated 6–13 h per day for 3 days. Modified from Knight *et al.* (1981).

RNA polymerase. As shown in Figure 5 ribavirin monophosphate will block inosine monophosphate (IMP) dehydrogenase and thus deplete the GTP pool and possibly also interact with the formation of cap structures on cellular mRNA (Goswami *et al.*, 1979). Functional cap structures from cellular mRNA are required as primers for influenza mRNA synthesis. These reactions seem to be competitive but it is not clear if the concentration of the competing substrates are sufficient and variable enough to influence in *in vivo* effect of ribavirin. A suppression of dTTP formation is also observed in cells (Drach *et al.*, 1981).

In mice, ribavirin given orally has a good therapeutic activity against influenza but, clinically, oral ribavirin has not been effective against influenza. However, ribavirin given as an aerosol is active clinically against influenza A infections as shown in Table IV. In contrast to amantadine and rimantadine, ribavirin is also active against influenza B infections and aerosol treatment has shown therapeutic effects as indicated in Table V and Figure 6. Interestingly ribavirin has also shown (Figure 7) therapeutic effects against respiratory syncytial virus infections in children. Apart from lung function improvement there was also a decrease in virus shedding. Since ribavirin is a ribonucleoside analogue the risk for mutagenic effects due to incorporation into DNA ought to be small as long as the modified base is not transferred

Table V. Clinical effect of ribavirin aerosol against influenza B

	Temperature (°C)			
	Day 0	Day 1	Day 2	Day 3
Treated ($N=11$)	39·2	38·5	37·8	37·3
Control ($N=10$)	39·0	39·1	38·3	37·7
t-test one-tailed	NS	0·02	0·028	NS

Patients were treated 3 to 4 h per day for 3 days. From McClung *et al.* (1983) with permission.

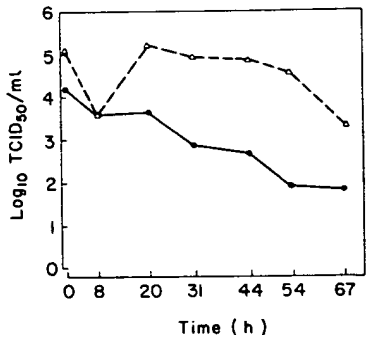


Figure 6. Clinical effect of ribavirin aerosol against influenza B. The aerosol was given 3×4 h per day starting less than 24 h after the first symptoms. Virus titres (50% tissue culture infectious doses [$TCID_{50}$] per ml of nasal secretion) of treated and control patients. From McClung *et al.* (1983) with permission. --△--, Control; —●—, treatment.

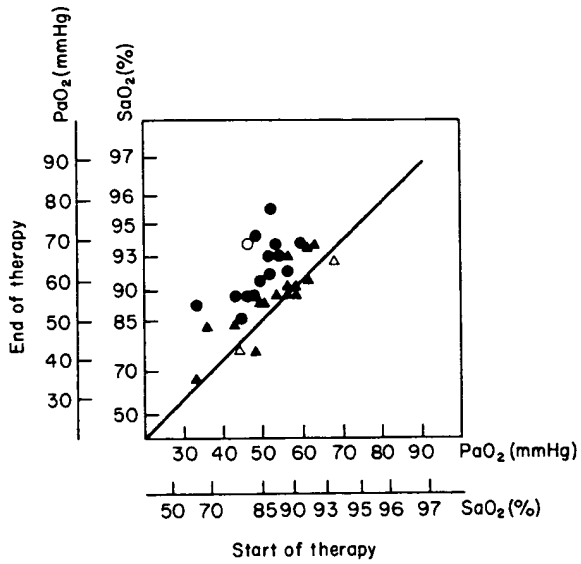
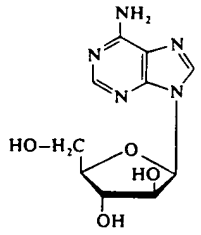


Figure 7. Clinical effect of ribavirin aerosol against respiratory syncytial virus infection in infants determined as arterial blood-gas levels at the beginning and end of therapy with ribavirin or placebo. The diagonal represents the line of identity, i.e. no change in values over the course of therapy. SaO_2 denotes arterial oxygen saturation and PaO_2 arterial oxygen tension. All infants were breathing room air except those three indicated by open symbols. The aerosol was given continuously for 3 to 4 days. From Hall *et al.* (1983) with permission. , Ribavirin patients, ▲, placebo patient, △, ○, on oxygen.

to a deoxyribose moeity or causes changes in the deoxyribonucleotide pools. However, some incorporation into DNA seems to occur and ribavirin has been reported to have teratogenic effects in rodents but not in primates. Different aspects of the biological effects and pharmacology of ribavirin have been reviewed by Sidwell, Robins & Hillyard (1979) and Smith & Kirkpatrick (1980).

Vidarabine, ara-A

Figure 8 shows the structure of ara-A. It has an adenine base attached to an arabinose sugar. Although ara-A has been studied for a rather long time its mechanism of action is still unclear. It is possible that several modes of action are involved and some are indicated in Figure 9. ara-A is phosphorylated by cellular enzymes and is incorporated in both cellular and herpesvirus DNA (Pelling, Drach & Shipman, 1981). There is some specificity since the triphosphate is a better inhibitor of herpesvirus DNA polymerase than of cellular DNA polymerase (Müller, Zahn & Falke, 1978, Drach & Shipman, 1977). The importance of normal substrates participating in the competitive reactions leading to an incorporation of ara-AMP into DNA is not clear, nor are the consequences of incorporation into cellular DNA. The therapeutic effects of ara-A and its more soluble monophosphate have been discussed elsewhere (Whitley, this volume) and will not be repeated. In contrast to its low activity against herpesviruses in cell culture, ara-A is surprisingly active both against herpes keratitis



9-β-D-Arabinofuranosyladenine-
(ara-A)

Figure 8. 9-β-D-Arabinofuranosyladenine (ara-A, vidarabine).

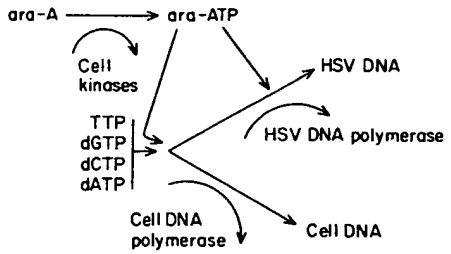


Figure 9. ara-A, mechanisms of action. ara-ATP competes with dATP and is incorporated into DNA. It also shows some selectivity in the inhibition of herpesvirus DNA polymerase and possibly has other effects. It is extensively deaminated to ara-H, hypoxanthine.

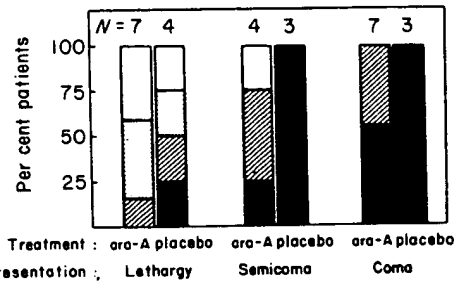


Figure 10. Clinical effect of ara-A against herpes encephalitis. The patients are grouped according to their state of consciousness at treatment initiation. □, No or minor sequelae; ▨, moderate sequelae; ▨, severe sequelae; ■, dead. From Whitley & Alford (1981) with permission.

and herpes encephalitis. It is still the only compound with demonstrated therapeutic effect against biopsy proven herpes encephalitis as exemplified in Figure 10. Buchanan & Hess (1980) have further reviewed the properties of ara-A.

Idoxuridine (IDU) and trifluorothymidine (TFT)

These two nucleoside analogues have structural similarities to thymidine as shown in Figure 11. This high degree of similarity makes it possible for both cytoplasmic thymidine kinase (TK) and herpesvirus TK to phosphorylate the analogues. As shown

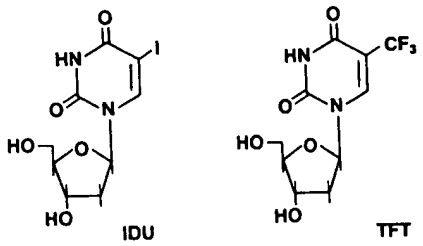


Figure 11. 5-Iodo-2′-deoxyuridine (idoxuridine, IDU) and 5-trifluoromethyl-2′-deoxyuridine (trifluorothymidine, TFT).

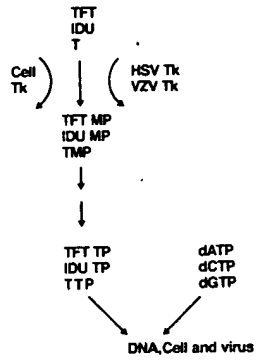


Figure 12. IDU and TFT, mechanism of action. IDU and TFT are phosphorylated in competitive reactions to triphosphates, which are incorporated into DNA, and this results in transcription and translation errors.

in Figure 12 they are further phosphorylated, probably by cellular enzymes, to triphosphates. IDU is also transformed to 5-iodouracil by thymidine phosphorylase and TFT-MP inhibits thymidylate synthetase resulting in a decreased pool of TMP and thus less competition during phosphorylation of TFT-MP. The incorporation of IDU and TFT monophosphates into viral and cellular DNA is at least partly the basis for their antiherpes activity. Since there is little specificity in this incorporation, these antiviral drugs can only be used where cellular DNA synthesis is minimal. The presence of nucleoside analogues in DNA will result in effects on transcription and translation (Otto, Lee & Prusoff, 1982). The HSV and VZV infected cells will contain more TK than uninfected cells and contribute to some selectivity and this might be one reason why TK-HSV is somewhat resistant to IDU. The incorporation of IDU and TFT monophosphates into DNA in uninfected cells is extensive and the restriction of the compound to topical use against herpes keratitis seem sensible. Furthermore at therapeutic concentrations, TFT, but not IDU, causes sister chromatid exchange in human cells (Cassiman *et al.*, 1981). IDU and TFT have been reviewed by Prusoff *et al.* (1979) and Heidelberger & King (1979).

Bromovinyldeoxyuridine (BVDU) and 2′-fluoro-5-iodo-arabinofuranosyl-cytosine (FIAC)

The halogenated nucleoside analogues BVDU and FIAC (Figure 13) have very high antiviral activity in cell cultures and are much more selective in their action than IDU and TFT but their mechanism of action is also based on a structural similarity to thymidine and deoxycytidine. BVDU and similar structures have been discussed elsewhere (De Clercq, this volume) and will only be commented upon briefly here.

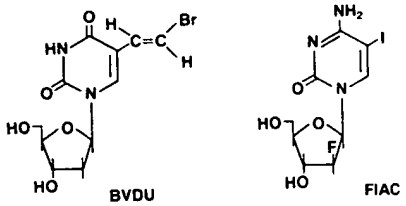


Figure 13. (E)-5-2-bromovinyl-2′-deoxyuridine (BVDU) and 2′-fluoro-5-iodo-l-β-D-arabinofuranosyl-cytosine (FIAC).

A possible mechanism of action is shown in Figure 14. Both compounds have a high selectivity in the first step and are phosphorylated by HSV and VZV TK much more effectively than by cellular kinases. The details of the further phosphorylation to triphosphates are less well known but all steps are probably competitive reactions. Both BVDU and FIAC show a selective inhibition of HSV DNA synthesis as compared to cellular DNA synthesis (Larsson & Öberg, 1981). However, both BVDU-MP and FIAC-MP, and its metabolite FMAU-MP (FMAU = 2′-fluoro-5-methyl-arabinofuranosyluracil) are incorporated into DNA which can be observed for BVDU as a shift in density of the viral DNA band in CsCl gradients (Larsson & Öberg, 1982), shown in Figure 15, and in the ability of FIAC-TP to support cell-free herpesvirus DNA synthesis in the absence of dCTP (Ruth & Cheng, 1981; Allauden *et al.*, 1982). FIAC seems to be extensively metabolized to FMAU before incorporation into

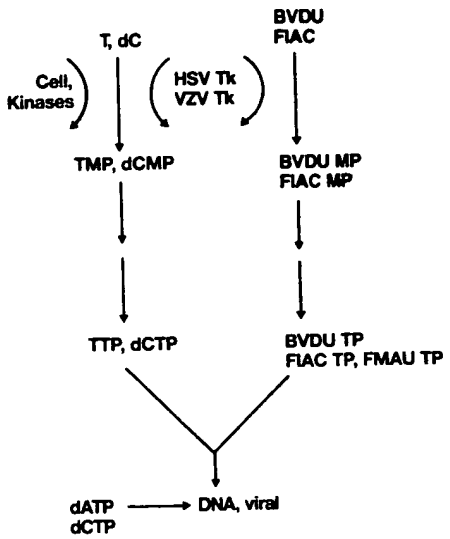


Figure 14. BVDU and FIAC, mechanism of action. Both compounds are first selectively phosphorylated by HSV and VZV TK and then further phosphorylated to triphosphates. These reactions are probably competitive. FIAC is to a large extent metabolized to 2'-fluoro-5-methyl-1-β-D-arabinosyluracil (FMAU). The triphosphates are probably competitive inhibitors and also incorporated into DNA.

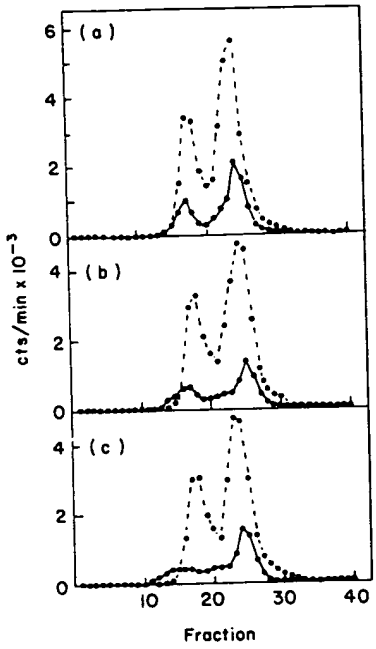
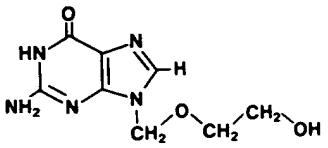
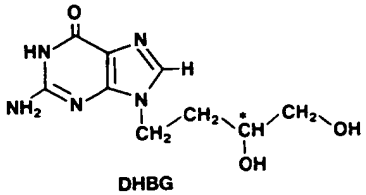


Figure 15. Effect of BVDU on DNA synthesis in HSV-1 infected cells. Density of DNA synthesized in HSV-1 infected Vero cells in the presence of BVDU. ●——●, [^{32}P]Orthophosphate-labelled DNA from infected cells treated with BVDU, ●– –●, [^{3}H]thymidine-labelled DNA from infected cells not treated with BVDU. (a) 0·025 μM BVDU, (b) 0·050 μM BVDU, (c) 0·10 μM BVDU. From Larsson & Öberg (1982) with permission.

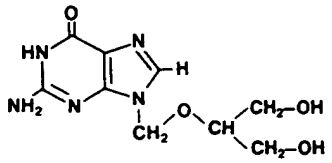
DNA. Incorporation of metabolized FIAC has also been shown to occur into the DNA of the small intestine in uninfected mice (Grant *et al.*, 1982). Since BVDU and FIAC, at least to a low extent, are likely to be phosphorylated even in uninfected cells and probably also be incorporated into cellular DNA, the biological consequences of this possibility should be considered. The modes of action of BVDU and FIAC as antiherpes agents are not quite clear but inhibition of viral DNA polymerase as well as incorporation into viral DNA resulting in defective mRNA and thus non functional proteins are probably of importance. The extent of incorporation of BVDU-MP into HSV DNA correlates to the antiviral activity and the incorporation also results in a labile DNA as revealed by an increased number of single strand breaks observed in alkaline sucrose gradients (Manchini *et al.*, 1983). The implication of a competitive mode of action in the different phosphorylation steps will be discussed later. The antiviral activity of BVDU has been reviewed by De Clercq (1983) and that of FIAC by Fox *et al.* (1982).



ACV



DHBG



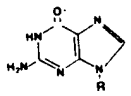
DHPG

Figure 16. 9-(2-Hydroxyethoxymethyl)-guanine (acyclovir ACV), 9-(3,4-dihydroxybutyl)guanine (DHBG), 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine (DHPG).

Acyclovir (ACV) dihydroxybutylguanine (DHBG) and dihydroxypropoxymethylguanine (DHPG)

The three guanine derivatives, ACV (for review see Elion, 1980), R-DHBG (Larsson *et al.*, 1983*a*) and DHPG (Smith *et al.*, 1982; Ashton *et al.*, 1982) shown in Figure 16, can, surprisingly, be phosphorylated by herpesvirus TK. As shown in Table VI several guanine derivatives can be selectively phosphorylated by HSV TK and some

Table VI. Phosphorylation and inhibition of HSV-1 plaque formation by some acyclic guanosine derivatives

	Thymidine kinase HSV-1 K_iμM	Velocity %	Vero K_iμM	HSV-1 plaque formation ED_{50}μM
CH_2-CH_2-CH-CH_2OH(*R*)-DHBG OH	1,5	73	>250	2
CH_2-CH_2-CH-CH_2OH (*S*)-DHGB OH	1,7	46	>250	13
CH_2-CH_2-CH-CH_2-OH (*RS*)-DHBG OH	1,5	75	>250	6
CH_2-CH-CH_2-CH_2OH (RS) OH	19	17	>250	64
$CH_2CH_2CH_2$-CH-CH_2OH (RS) OH	43	59	>250	140
CH_2-CH_2-CH-CH_2-CH_2OH (RS) OH	44	45	>250	43
CH_2-CH_2-CH_2-CH_2-OH-CH_2-OH (RS) OH	>250	<5	>250	82
CH_2-CH_2-CH_2-CH_2OH EHB 682	2,1	10	<250	3
CH_2O-CH_2-CH_2OH ACV	173	27	<250	0,3
Thymidine	0,41 (K_m)	100	1,31 (K_m)	

(From A. Larsson, personal communication.)

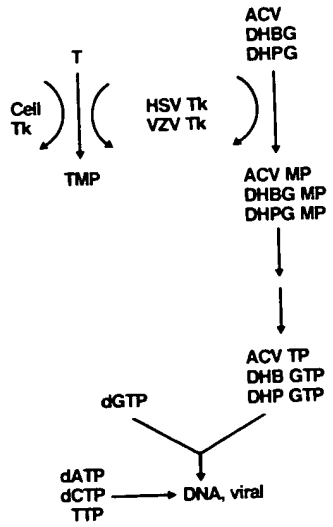


Figure 17. ACV, DHBG and DHPG, mechanism of action. The nucleoside analogues are selectively phosphorylated by HSV and VZV TK and then further phosphorylated to triphosphates. These reactions are probably competitive. The triphosphates of ACV and DHPG show a selective inhibition of viral DNA polymerase and are also incorporated into DNA.

of them also show antiviral activity against HSV in cell culture. The ability to be phosphorylated to monophosphates is a necessary but not sufficient property for antiviral activity in cell culture. Similar results have been reported for other guanine

derivatives by Keller *et al.* (1981). The likely modes of action of ACV, DHBG and DHPG are illustrated in Figure 17. The mechanism of action of ACV is best known and is shown in more detail in Figure 18. In the first phosphorylation there is a competition with thymidine and later with dGMP (Miller & Miller, 1980), possibly dGDP (Miller & Miller, 1982) and in the last step with dGTP. ACV-TP is both an inhibitor and an alternative substrate for the viral DNA polymerase (Furman *et al.*, 1979). Since ACV has only one hydroxyl group available for phosphodiester bond formation it will terminate a DNA chain. DHPG contains two hydroxyl groups and can be incorporated in such a manner as to allow elongation. Moreover, incorporation into DNA has been reported (Cheng *et al.*, 1983). It is presently unclear if DHBG, which also contains two hydroxyl groups, is incorporated into DNA. The non-competitive inhibition of herpesvirus DNA polymerase by terminally incorporated ACV (Figure 18) is interesting and could mean that the nucleoside analogue

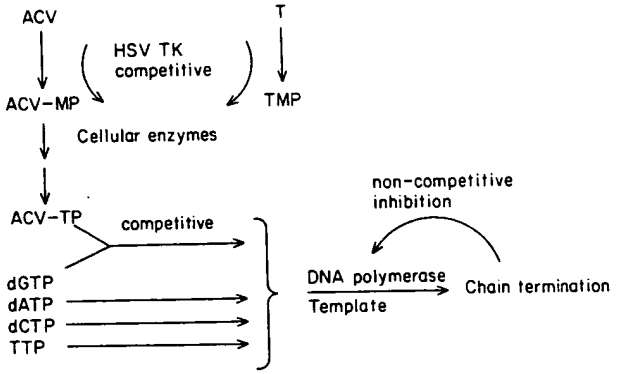


Figure 18. ACV, mechanism of action. Apart from the competitive steps, shown in Figure 17 the terminal incorporation into DNA results in a non-competitive inhibition of the polymerase activity.

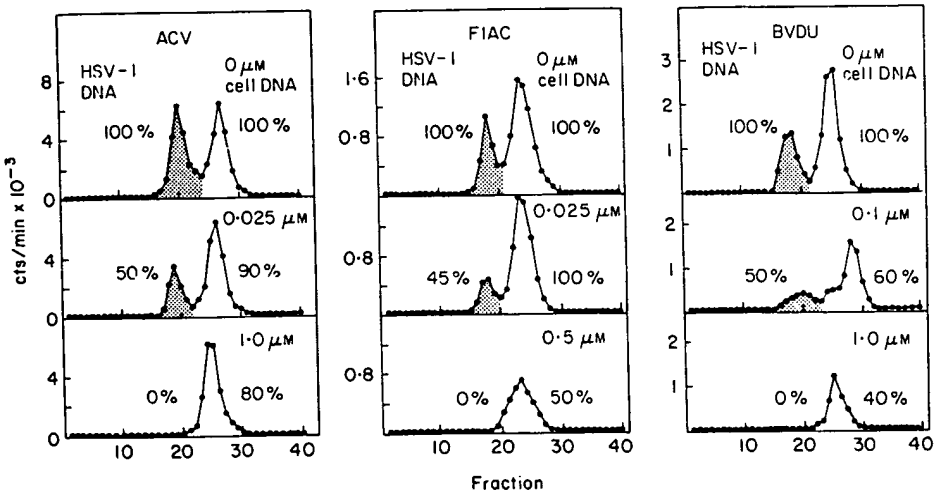


Figure 19. Selectivity of inhibition of cellular and HSV DNA synthesis in HSV-1 infected Vero cells by ACV, FIAC and BVDU. Viral DNA (hatched area) and cellular DNA were separated in CsCl gradients. From Larsson & Öberg (1981) with permission.

could not be removed by the nuclease activity associated to herpesvirus DNA polymerase (Derse *et al.*, 1981).

The selectivity of ACV against viral DNA synthesis is easily seen when DNA from HSV infected and ACV treated cells is banded in CsCl gradients as shown in Figure 19, and ACV is a more selective inhibitor of viral DNA synthesis than FIAC or BVDU.

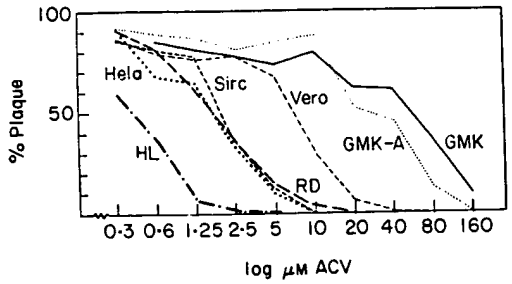


Figure 20. Effect of ACV against plaque formation by one strain of HSV-1 in different cell cultures. From Harmenberg *et al.* (1980) with permission.

Comparisons

When the antiviral activity of a nucleoside analogue is tested against one strain of herpesvirus but in different cell cultures (Figure 20) a 50% inhibition of HSV-1 plaque formation is noted at concentrations widely different for different cells (Harmenberg, Wahren & Öberg, 1980). This type of variation can also be seen for other nucleoside analogues requiring HSV TK for phosphorylation. At least part of the explanation for these differences is the thymidine content of the cells. The cells where ACV has the best antiviral activity against HSV have the lowest thymidine content (Harmenberg, 1983). There are also differences in kinase activity (Harmenberg, Källander & Gronowitz, 1984) but the thymidine effect is probably of more importance.

Table VII. Reversal of antiherpes activity by thymidine in cell culture

μM dThd added	Concentration of drug causing (50%inhibition (μM)			
	Acyclovir	(*R*)-Dihydroxybutyl-guanine	Bromovinyl-deoxyunidine	Foscarnet
0	0·5	1·0	0·2	48
5	1·0	2·4	2·0	–
10	2·3	2·8	2·9	–
25	4·4	3·9	7·2	42
50	6·7	6·3	13	42
100	14	11	22	45
Ratio $\frac{0\ \mu M\ dThd}{100\ \mu M\ dThd}$	28	6	110	1

The 50% inhibition values were determined graphically from dose-response curves where 5 different concentrations of test compounds were used. The values are mean values from 3 different experiments. Modified from Larsson, Brännström & Öberg (1983*b*).

Reversal of the inhibition due to nucleoside analogues can, in cell culture, be achieved by addition of thymidine (Larsson, Brännström & Öberg, 1983*b*). This is shown in Table VII for a few compounds. It is evident that the sensitivity to reversal differs between different nucleoside analogues in a manner reflecting their affinity to HSV TK. The kinetics of this reversal can be determined in cell culture analogous to enzyme kinetic studies and Figure 21 shows the reversal kinetics for ACV, DHBG

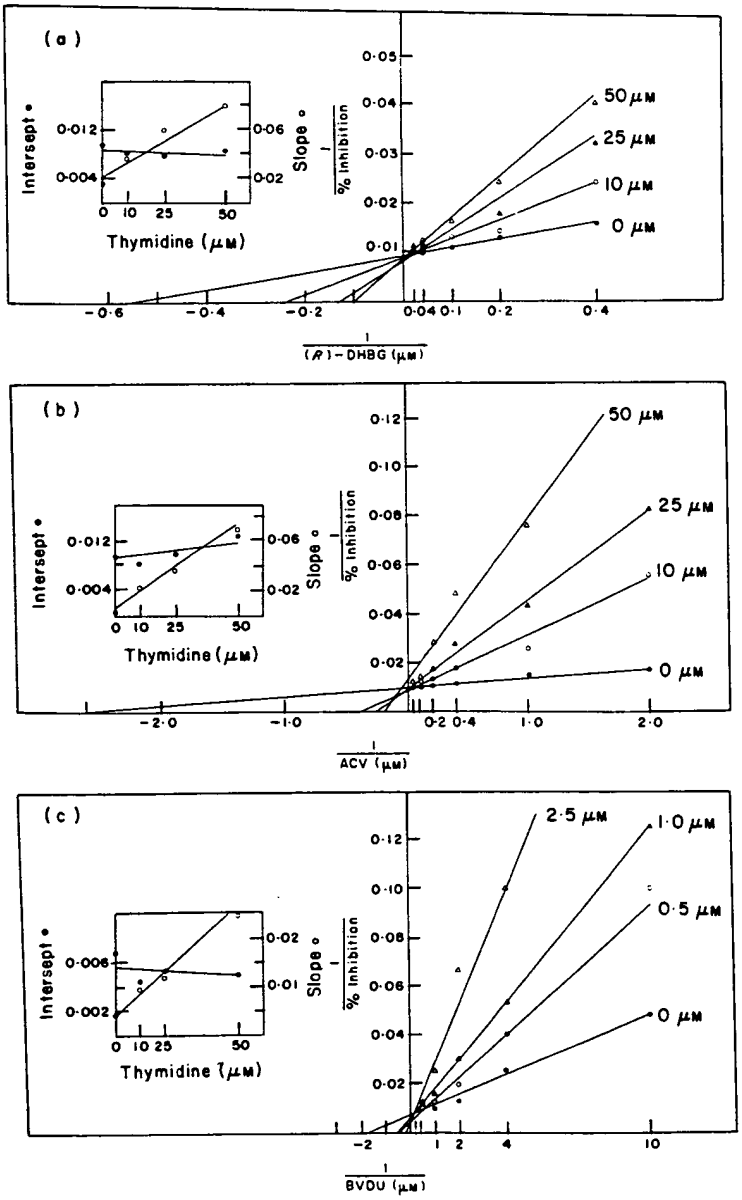


Figure 21. Inhibition of HSV-1 plaque formation by antiherpes drugs, kinetic patterns of reversal by thymidine. Antiherpes drugs were used as the variable substrates and the inhibition of HSV-1 strain C42 plaque formation in Vero cells was measured at different thymidine concentrations. (a) (*R*)-DHBG, (b) ACV, (c) BVDU. After Larsson *et al.* (1983*b*).

Table VIII. Phosphorylation of acyclovir and dihydroxypropoxymethylguanine in primary rabbit kidney cells

Drug	HSV-1 infection	nmoles/10^7 cells/6 h N	NMP	NDP	NTP
DHPG	−	0·58	0·10	0·11	0·19
DHPG	+	0·34	0·21	1·16	10·29
ACV	−	0·07	0·07	0·14	0·12
ACV	+	0·28	0·07	0·20	1·47

N = DHPG, or ACV, MP, DP and TP are mono-, di- and triphosphates, respectively, of the nucleoside analogues. After Germershausen *et al.* (1983) with permission.

and BVDU. DHBG has 100 times higher affinity than ACV to the viral TK and its effect is less easily reversed by thymidine. Since the thymidine content in skin is rather high, reaching 10 to 20 μM in guinea pigs, (Harmenberg, 1983), this would influence the therapeutic effect *in vivo*. The effective reversal of the inhibition by BVDU, which has a high affinity for the viral TK, is probably also due to increased concentrations of the TDP and TTP, which are competitive substrates. Even if the thymidine content of cells influences the antiherpes activity of some nucleoside analogues this is certainly not the only factor determining the therapeutic effect. A comparison of the phosphorylation of ACV and DHPG in primary rabbit kidney cells reveals (Table VIII) that the phosphorylation to mono-, di- and triphosphate is more efficient for DHPG than for ACV (Germershausen *et al.*, 1983). However, the differences are not as large as expected from experiments using purified herpesvirus TK and hog brain GMP kinase studying the two first steps of phosphorylation (Ashton *et al.*, 1982).

Table IX illustrates the relative efficacy, on a molar basis and using the best possible vehicles, of a few compounds tested in different animal models of HSV-1 infections. It is evident, as also reported previously (Alenius & Öberg, 1978; Alenius *et al.*, 1982), that the cell culture efficacy cannot be used to predict the relative therapeutic effect in animals, even when the same strain of HSV is used. This is perhaps not surprising since the drugs in different animal species could be expected to differ

Table IX. Efficacy rank in animal HSV-1 models

Cell culture ID_{50}, μM	Cutaneous topical	Genital topical	Keratitis topical	Encephalitis oral	Systemic ip	Systemic oral
BVDU 0·03	Foscarnet	Foscarnet	BVDU	ara-A	(*R*)-DHBG	(*R*)-DHBG
ACV 0·3	(*R*)-DHBG	ACV	ACV	ACV	ACV	ACV
(*R*)-DHBG 4	ACV	BVDU	(*R*)-DHBG	(*R*)-DHBG	Foscarnet	ara-A
ara-A 20	BVDU	(*R*)-DHBG	ara-A	Foscarnet		Foscarnet
Foscarnet 30	ara-A	ara-A	Foscarnet			

(From A.-C. Ericson, personal communication.)

as regards uptake, degradation and distribution to the infected tissue. The comparative effects of DHPG have not yet been reported in identical models but published results show a high activity when given orally to mice with mucocutaneous or systemic HSV infections (Field *et al.*, 1983). Since the activation and metabolism of nucleoside analogues involves several cellular enzymes, which might differ between mice and man, a determination of ID_{50} in cell culture and the actual concentration of the drug and competing substrates in the infected tissue might possibly be used to predict efficacy in man.

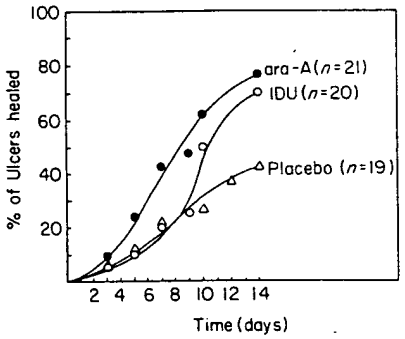


Figure 22. Cumulative frequency curves showing healing rates in three groups of patients with herpes keratitis. The groups (out-patients) were treated with placebo ointment, 3% ara-A ointment or 0·5% IDU ointment four times a day. After Markham *et al.* (1977)

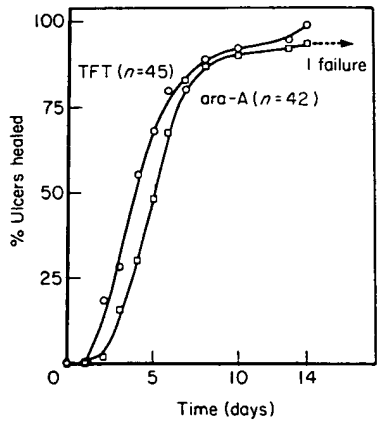


Figure 23. Cumulative frequency graph of rates of healing of dendritic herpes ulcers under treatment with 3% ara-A ointment or 1% TFT eye drops five times daily. After Coster *et al.* (1976).

Only one type of viral infection in humans, herpes keratitis, has been the target of studies comparing the efficacy of several nucleoside analogues and Figures 22 to 26 show results from some of these studies. The reference point has been IDU which was reported earlier to give a therapeutic effect against herpes keratitis. In rabbits with a primary herpes keratitis, IDU has an excellent therapeutic effect but in recurrent clinical cases the effect is not very impressive and in one well-controlled study by Markham *et al.*, (1977) no significance was reached although there was a tendency

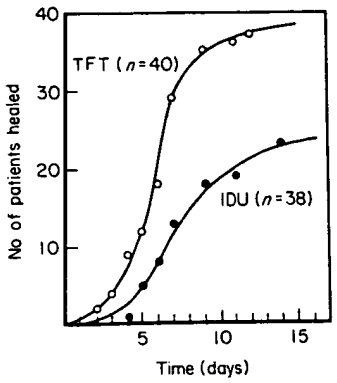


Figure 24. Healing of herpes ulcers treated with TFT and IDU. Cumulative frequency of days required to heal. IDU was given as 0·1% eye drops and TFT as 1% eye drops, 1 drop five times daily. After Wellings *et al.* (1972).

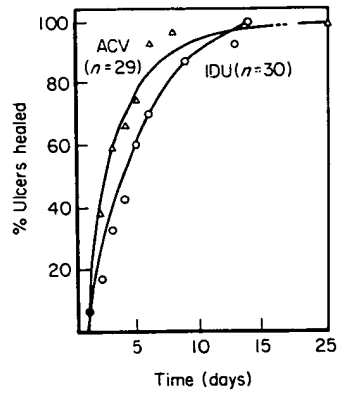


Figure 25. Cumulative frequency graph of the time taken to heal 59 herpetic corneal ulcers (54 dendritic, 5 geographic) treated with 3% ACV ointment or 1% IDU ointment. After Coster *et al.* (1980).

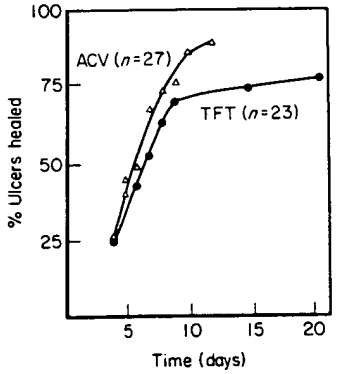


Figure 26. Cumulative frequency distribution of the time taken to heal dendritic herpes keratitis in 27 patients given 3% ACV ointment and 23 patients given 2% TFT ointment 5 times daily. After Lau *et al.* (1982).

indicating clinical efficacy. Debridement, steroids and other types of concurrent treatment probably influence the result of treatment more than the actual choice of nucleoside analogue, although these differ considerably in cell culture efficacy against HSV. Since most nucleoside analogues in controlled studies have given therapeutic effects similar to the reference compound IDU there is some uncertainty as to their true therapeutic potential, and although herpes keratitis is possibly the herpes infection most similar to a cell culture experiment there is still no obvious correlation between clinical benefit and cell culture efficacy.

In other herpes infections where penetration, oral uptake, metabolism and interfering nucleosides and nucleotides are even more likely to make a prediction of efficacy difficult, there are no results from controlled clinical trials comparing different nucleoside analogues.

General aspects of nucleoside analogues

Table X lists a few of the many important aspects on nucleoside analogues one has to consider in selecting them as antiviral drugs. Only those properties that are directly connected to this type of chemical structure are listed. Properties such as general toxicology, pharmacology and pharmacokinetics are not considered as they are of general importance and not specifically connected to nucleoside analogues. Furthermore, exact information in these areas is often absent in the literature.

The first point in Table X is of course trivial but none the less important because the more specificity the less problems with, for example, incorporation into cellular DNA in uninfected cells. However, it would be naive to think of any inhibitor as absolutely specific and one has to consider the effects of incorporation of nucleoside analogues into cellular DNA in the non-infected cell (see Table VIII). There is also the possibility that nucleoside triphosphates could pass through tight junctions from an infected to an uninfected cell. If an incorporation into DNA results in the death of the cell it is probably of no serious consequence as long as it happens only in a few cells. If the incorporation results in a permanent localization of a nucleoside analogue in a DNA strand this might have consequences for base-pairing, methylation, recognition by enzymes, etc., all of which possibly could result in mutagenic and carcinogenic effects.

Nucleoside analogues which seem to carry less risk than others for carcinogenic potential are those with a normal base, such as ACV, DHBG and DHPG, and a metabolic transfer of the base to a new sugar by nucleoside phosphorylases should not be of any concern. A nucleoside analogue, such as ACV, terminating a growing DNA chain might have less risk of causing trouble than one incorporated internally although the terminal incorporation will lead to fragmentation of DNA.

Of course, all new drugs of this type are evaluated for carcinogenic effects in long term experiments in mice and rats and also in different cell culture systems. However, these are systems with enzymes possibly differing from those of man in their ability to recognize nucleoside and nucleotide analogues and extrapolation from mice to man might be especially dangerous with compounds involving the action of several cellular as well as viral enzymes. A safety evaluation should include comparative studies in human cells and rodent cells to make a prediction for possible carcinogenic effects in humans.

Since the nucleoside analogues used as antiherpes drugs are substrate analogues

Table X. The ideal antiviral nucleoside analogue

(1)	High selectivity for viral function(s)
(2)	Normal base
(3)	Not incorporated into DNA
(4)	If incorporated: Not mutagenic or carcinogenic
(5)	Un- or non-competitive inhibitor/substrate
(6)	If competitive: A high affinity to enzyme

in several reactions of importance for the antiviral effect, the ability of the nucleoside analogues to compete with the natural substrates, and the concentrations of these substrates *in vivo* will be of importance for efficacy. This is easily forgotten and ranking the antiviral activity of nucleoside analogues according to efficacy in a cell culture assay could be misleading if the intention is to predict *in vivo* efficacy. The *in vivo* efficacy of a compound is, of course, influenced by several other factors such as degradation and pharmacokinetics.

We have just begun to realize the difficulty in predicting clinical efficacy of nucleoside analogues and possible side effects due to incorporation into DNA, but the next years will certainly provide us with a much deeper understanding of these questions and help us to design better antiviral drugs.

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